



Replacement Sheet

MKK4 (cDNA accession number: AB015315)

*Wild-type cDNA sequence from **start** to **end** codons
(1101 base pairs including the end TAG):*

atgagaccgattcaatgcctcaggagttccgttccggtaaaagccgtccccgtcg
ccgtcctgatcttaccttaccgcctcaacgcgtatgttctctcgctgtacctcttc
ctctcccacctacttccgtgggtccgggtcttagtggatctgcgcgtcttctgg
ggttcggcgtcttcaacgaacactaacagctccatagaagcgaagaactattcggatt
agttagaggttaaccgtatcgaaagcggagcaggtggaaacggatataaaagtgattcacc
gtccgagttctcgcttatatgcacttaaggatatacgtaaccacgaggagacttg
agacgtcagatctgttagagagatcgagatttacgagatgtgaatcatccaaacgttgc
gaaatgtcagatctgttagagatcgagatccaggatgtttgcttgcgttatgg
ataaaaggatctttagaaggtagctcatgtgtggaaagagcaacaattagctgttatct
cgtagattcttagtggtagcttgcataccatcgatgtttatcgatcatcgatcatcgat
caaaccatcgaatctttgataaaactctgctaaaaacgttaagattgtgatattggag
tttagtagatcttggctcagactatggatccgtgtatctgtttgatccatcgatcatcgat
tatatgagtccctgagaggatataactgtatgtttgatccatcgatcatcgatcatcgat
tggagatattggagcttaggtgttagcattttggagatgtttacttggggaggatcttgc
tccctgtgatagacaaggtagttggcttagtcttatgtgtgcattgtatgtctcag
cctccagaagctccagcgtactgcgtcgccggatgttccggatattatctcgatcatcgat
gcagagagaaccggggaaaaggaggagtgctatgcagctattgcagcatccttcata
taagagcaagtcccgagccagaacaggctcctcagaatctacatcaactcttcct
cctcgatctgttcctcgatcttcataaccatcgat (SEQ ID NO.:3)

Wild-type protein sequence (366 aminoacids):

MRPIQSPPGVSPVKSRRPDLTLPLPQRDVSLAVPLPLPPTSGGSGGSSGSAPSSG
GSASSTNTNSSIEAKNYSSDLVRGNRIGSGAGGTYVKVIHRPSSRLYALKVIYGNHEETV
RRQICREIEILRDVNHPNVVKCHEMFDQNGEIQVLLFMDKGSLEAHVWKEQQLADLS
RQILSGLAYLHSRHIVHRDIKPSNLLINSAKNV рIADFGVSRILAQTMDPCNSSVGTIA
YMSPERINTDLNQGKYDGYAGDIWSLGVSILEYLGRFPFPVSRQGDWASLMCAICMSQ
PPEAPATASPEFRHFISCLQREPGKRRSAMQLQHPFILRASPQNRSPQNLHQLLPP
PRPLSSSSSPTT (SEQ ID NO.:4)

FIGURE 4

Replacement Sheet

Mutations rendering MKK4 constitutively active:

- modify Threonine (T) 224 to Aspartic Acid (D) by changing codon ACT into GAT
- modify Serine (S) 230 to Glutamic Acid (E) by changing codon TCA into GAA

The mutations were done by PCR using the primers (mutated base pairs in lower case, both are from 5' end to 3' end):

CTTGGCTCAGgaTATGGATCCGTGTAATgaATCTGTTGGAAC
TCCAAACAGAttcATTACACGGATCCATAccCTGAGCCAAG (SEQ ID NO.3)

So the sequences after mutations are:

MKK4act mutant sequence from ~~start~~ to ~~end~~ codons
(1101 base pairs including the end TAG):

MKK4act mutant protein sequence (366 aminoacids):

MRPIQSPPGVSVPVKSRRPDLTLPLPQRDVSLAVPLPLPPTSGGSGGSSGAPSSGGSASSTNTNSSIEAKNYSSDLVRGNRIGSGAGGTVYKVIHRPSSRLYALKVIYGNHEETVRRQICREIEILRDVNHPNVKCHEMFDQNGEIQVLLFMDKGSLEAHVWKEQQQLADLSRQILSGLAYLHSRHIVHRDIKPSNLLINSAKNVKIADFGVSRLAQMDPCEESVGTIAYMSPERINTDLNQGKYDGYAGDIWSLGVSILEFYLGRFPFPVSRQGDWASLMCAICMSQPPEAPATASPEFRHFISCLQREPGBKRRSAMQLQHPFILRASPQNRSPQNLHQLLPPP
RPLSSSSSPTT (SEQ ID NO.:7)

FIGURE 5

Replacement Sheet

MKK5 (cDNA accession number: AB015316)

Wild-type cDNA sequence from **start** to **end** codons
(1047 base pairs including the end TAG):

atcgaaaccgattcaatctccttctggagtagcttcacctatgaagaaccgttacgcaa
acgtcctgacctaagcttaccactcccacaccgcacgtcgctctcgccgtacctctcc
ctctcccacctccttctccttcatccgctccggcgtctcctccgcataacc
aacatctccgcgctaaaagcttatccgagctagaacgagtgaaaccgaatcggaaagcgg
agccggaggaacggttacaaagaatccacactccgacgtcacgtccttcgctctca
aagtgatttacggaaaccacgaagataccgtgagacgtcagatctgttagagagatcgag
atcttaagaagtgttgcataccaaacgttgcataatgtcacgatatgtttgatcataa
cggtgagatccaggtttgcattgcatttatggatcaaggatcttgcataaggagctcata
tatggcaagaacaggaattagctgatctctcgtcagatttttagtggattagctt
cttcatcgtcgtcatatcgatcgatccaaaccttcgaatctccttataaactc
agctaaaaatgtgaaaattgctgattttgggtgagtaggatcttggcacaacaaatgg
atccttgcataatgcattgcgttactattgcattatgcgtcagatcttgcataatgc
gatttgcataatgcgttgcgttacgtggatgcgtggagatgtttggagtttagtgc
tatcttggagtttactggggagggttgcatttgcgtgagtagacaaggatgttgc
ctagtcttatgtgtctattgtatgtctcagccacctgaagctccggctacggcgtct
caggagttcgtcacttgcatttgcatttgcgttgcatttgcgttgcatttgcatttgc
agctcaacacagctttgcagcatccttcataacttgcatttgcatttgcatttgc
qtcaaatgttgcgttgcatttgcatttgcatttgcatttgcatttgcatttgcatttgc (SEQ ID NO.:8)

Wild-type protein sequence (348 aminoacids):

MKPIQSPSGVASPMKNRLRKRPDLSLPLPHRDVALAVPLPLPPPSSSSSAPASSAIST
NISAALKSELERVNRIGSGAGGTVYKVIHTPTSRPFALKVIYGNHEDTVRRQICREIE
ILRSVDHPNVVKCHDMFDHNGEIQVLLEFMDQGSLEGAHIWQEQLADLSRQILSGLAY
LHRRHIVHRDIKPSNLLINSAKNVKIADFGVSRILAQTMDPCNSVGTIAYMSPERINT
DLNHGRYDGYAGDVWSLGVSILEFYLGRFPFAVSQRQGDWASLMCAICMSQPPEAPATAS
QEFRHFVSCCLQSDPPKRWSAQQLLQHPFILKATGGPNLRQMLPPRPLPSAS (SEQ
ID NO.: 9)

FIGURE 6

Replacement Sheet

Mutations rendering MKK5 constitutively active:

- modify Threonine (T) 215 to Glutamic Acid (E) by changing codon ACA into GAA
- modify Serine (S) 221 to Glutamic Acid (E) by changing codon TCA into GAA

The mutations were done by PCR using the primers (mutated base pairs in lower case, both are from 5' end to 3' end):

CTTGGCACAAgaAATGGATCCTTGTAAgaATCTGTTGGT
ACCAACAGATTcATTACAAGGATCCATTcTTGTGCCAAG (SEQ ID NO.:10)

So the sequences after mutations are:

MKK5act mutant cDNA sequence from start to end codons (1047 base pairs including the end TAG):

atgaaaccgattcaatctccttggagtagcttcacctatgaagaaccgttacgcaa
acgtcctgacctaagcttaccactcccacaccgcacgtcgctcgccgtacctctcc
ctctcccacccctcttctcatccgctccggcgttctcctcctcgtactcaacc
aacatctccgccctaaagctttccgagtagaacgaggtgaaccgaatcggaagcgg
agccggaggaacggttacaaaagtaatccacaccctgacgtcacgtcttcgctca
aagtgattacggaaaccacgaagataccgtgagacgtcagatcttgagagagatcgag
atcttaagatgttgatcatccaaacgtttgtgaaatgtcagatatgtttgtatca
cggtagatccaggttttgcttgagttatggatcaaggtcttttggaaggagctcata
tatggcaaacaggaattatgctgatctctctcgtcagatcttttgatggattagcttt
cttccatcgtcatatcgttcatcgtgatatcaaaacccttcgaaatctctttataaaactc
agctaaaaatgtgaaaattgctgatttggtggagtgaggatcttgggcacaaaatgg
atcctttgaaatgtgaaatctgtggtactattgctttattgaggtcctgagaggattaact
gatttgaaatcatggtcgttacgatggttatgctggagatttggagttaggtttag
tatcttgagtttacttgggagggttccttttctgtgagtacaaggtgattgggg
ctagtctttatgtgctatttgttgatgtctcagccacctgtaagctccggctacggcgct
caggatttcgtcactttgtttctgttgttacaggtgatcctctaagagatggtc
agctcaacagctttgcagcatcttcatactaaagctacccggtggtccttaatctcc
gtcaaaatgtgccccccctcgctttctgtcccttttgat (SEQ ID NO.:11)

MKK5act mutant protein sequence (348 aminoacids):

MKPIQSPSGVASPMKNRLRKPDSLPLPHRDVALAVPLPLPPSSSSAPASSAIST
NISAAKSLSELERVNRIGSGAGGTVYKVIHPTSRPFALKVIGNHEDTVRRQICREIE
ILRSVDHPNVVKCHDMFDHNGEIQVLFMDQGSLEGAHIWQEQELADLSRQILSGLAY
LHRRHIVHRDIKPSNLLINSAKNVKIADFGVSRILAQEMDPCNESVGTIAYMSPERINT
DLNHGRYDGYAGDVWSLGVSIEFYLGRPFAVSRQGDWASLMCAICMSQPEAPATAS
QEFRHFVSCCLQSDPPKRWSQQLLQHPFILKATGGPNLRQMLPPRPLPSAS (SEQ ID NO.:12)

FIGURE 7

Replacement Sheet

Alignment of MKK4 and MKK5 wild-type:

MKK4 MRPIQSPPGVSVPVKSRRPDLTLPLPQRDVSLAVPLPLPPTSGSGG
MKK5 MKPIQSPPGVASPMKNRLRKPDLSLPLPHRDVALAVPLPLPPPS-----

MKK4 SSGSAPSSGGSASSTNTNSSIEAKNYSDLVRGNRIGSGAGGTVYKVIH
MKK5 SSSSAPASS-SAISTNIS---AAKSLSELERVNRIGSGAGGTVYKVIHTP

MKK4 SSRLYALKVIYGNHEETVRRQICREIEILRDVNHPNVVKCHEMFQN
MKK5 TSRPFALKVIYGNHEDTVRRQICREIEILRSVDHPNVVKCHDMFDHN
GEI

MKK4 QVLLEFMDKGSLEGAHVWKEQQLADLSRQILSGLAYLHSRHHIVHRD
IKPS
MKK5 QVLLEFMDQGSLEGAHIWQEQELADLSRQILSGLAYLHRRHHIVHRD
IKPS

MKK4 NLLINSAKVKIADFGVSRILAQTMDPCNS
SSVGTIA
YMSPERINTD
LNQG
MKK5 NLLINSAKVKIADFGVSRILAQTMDPCNS
SSVGTIA
YMSPERINTD
LNHG

MKK4 KYDGYAGDIWSLGVSILEFYLGRFPVSRQGDWASLMCAICMSQP
PEAP
MKK5 RYDGYAGDVWSLGVSILEFYLGRFPFAVS
RQGDWASLMCAICMSQP
PEAP

MKK4 ATASPEFRHFISCLQREPGKRRSAMQLLQHPFILRASP
SQNRSPQNLHQ
MKK5 ATASQEFRHFVSCCLQSDPPKRWSAQQLLQHPFILKATG----GP-NLRQ

MKK4 LLPPPRPLSSSSPTT (SEQ ID NO.:13)
MKK5 MLPPPRPLPSAS---- (SEQ ID NO.:14)

FIGURE 8